The Keap1/Nrf2 pathway in health and disease

O'Connell, Maria A.; Hayes, John D.

Published in:
Biochemical Society Transactions

DOI:
10.1042/BST20150069

Publication date:
2015

Document Version
Peer reviewed version

Dual regulation of transcription factor Nrf2 by Keap1 and by the combined actions of β-TrCP and GSK-3

John D. Hayes[1,3], Sudhir Chowdhry[1], Albena T. Dinkova-Kostova[1] and Calum Sutherland[2]

[1]Jacqui Wood Cancer Centre,
Division of Cancer Research, and
[2]Division of Cardiovascular and Diabetes Medicine,
Medical Research Institute,
Ninewells Hospital and Medical School,
University of Dundee,
Dundee DD1 9SY,
Scotland,
United Kingdom

[3]Correspondence:e-mail, j.d.hayes@dundee.ac.uk; telephone +44 (0)1382 383182

Key words:
Nrf2, β-TrCP, GSK-3, PI3K, PKB/Akt, epidermal growth factor, keratinocyte growth factor

Word count:

Abstract, 234 words;
Main text, 2997 words.

Abstract
NF-E2 p45-related factor 2 (Nrf2) is a master regulator of redox homeostasis that allows cells to adapt to oxidative stress and also promotes cell proliferation. In this review we describe the molecular mechanisms by which oxidants / electrophilic agents and growth factors increase Nrf2 activity. In the former case, oxidants / electrophiles increase the stability of Nrf2 by antagonizing the ability of Kelch-like ECH-associated protein 1 (Keap1) to target the transcription factor for proteasomal degradation via the Cullin-3–based E3 ubiquitin ligase CRL^{Keap1}. In the latter case, we speculate that growth factors increase the stability of Nrf2 by stimulating phosphoinositide 3-kinase (PI3K)–protein kinase B (PKB)/Akt signaling, which in turn results in inhibitory phosphorylation of glycogen synthase kinase-3 (GSK-3), and in so doing prevents the formation of a DSGIS motif-containing phosphodegron in Nrf2 that is recognised by the β-transducin repeat-containing protein (β-TrCP) Cullin-1–based E3 ubiquitin ligase complex $SCF^{\beta\text{-}TrCP}$. We present data showing that in the absence of Keap1 the electrophile *tert*-butyl hydroquinone (tBHQ) can stimulate Nrf2 activity and induce the Nrf2-target gene *NAD(P)H:quinone oxidoreductase-1* (i.e. *NQO1*), whilst simultaneously causing inhibitory phosphorylation of GSK-3β at Ser-9. Together, these observations suggest that tBHQ can suppress the ability of $SCF^{\beta\text{-}TrCP}$ to target Nrf2 for proteasomal degradation by increasing PI3K–PKB/Akt signaling. We also propose a scheme that explains how other protein kinases that inhibit GSK-3 could stimulate induction of Nrf2-target genes by preventing formation of the DSGIS motif-containing phosphodegron in Nrf2.

Background

Four cap'n'collar (CNC) basic-region leucine zipper (bZIP) transcription factors have been described in mammalian species, designated nuclear factor-erythroid 2 p45 (NF-E2 p45), and NF-E2 p45-related factor 1 (Nrf1[1]), Nrf2 and Nrf3 [1]. They bind their cognate DNA sequences in the regulatory regions of target genes as heterodimers with the small musculoaponeurotic fibrosarcoma (Maf) bZIP proteins, MafF, MafG and MafK [2-4]. Expression of the NF-E2 p45 and Nrf3 transcription factors are restricted primarily to haematopoietic tissue and placenta, whereas Nrf1 and Nrf2 are widely expressed across mammalian tissues [5-7]. Each of the CNC-bZIP transcription factors is functionally distinct, as evidenced by the markedly different phenotypes observed upon their knockout in the mouse [8, 9].

Since its discovery in 1994 as a protein that binds a DNA sequence consisting of a tandem repeat of the activating protein 1 (AP1) site present in the β-globin gene locus control region [10], the work of Masayuki Yamamoto, Tom Kensler and many others has revealed that Nrf2 plays a central role in cancer chemoprevention and to be a master regulator of redox homeostasis [11-15]. More recently it has become apparent that Nrf2 can markedly influence intermediary metabolism by controlling carbon flux through the pentose phosphate pathway, inhibiting lipid synthesis, increasing β-oxidation of fatty acids, and supporting mitochondrial respiration [16-19].

Stimulation of Nrf2 activity by both oxidants / electrophiles and growth factors

A cardinal feature of Nrf2 is that its activity, and hence the expression of its target genes, is maintained at low levels under normal homeostatic conditions but increases rapidly in response to redox and electrophilic stressors as well as by stimulation by growth factors. The diverse biological effects of Nrf2 are exerted through its ability to mediate induction of genes that contain in their promoter regions an antioxidant response element (ARE[2], 5′-$^{A}/_{G}$TGA$^{C}/_{G}$NNNGC$^{A}/_{G}$-3′) [20] upon exposure to a wide spectrum of oxidants and soft electrophiles (see **Figure 1** for some examples) [21-23], or stimulation by epidermal growth factor [24, 25], fibroblast growth factor [26], insulin [27, 28], insulin-like growth factor [29], keratinocyte growth factor [30, 31], nerve growth factor [32], platelet-derived growth factor [33] or supply of glucose [34]. To date, approximately 250 genes that contain ARE sequences have been reported in mice and humans [35-39]. Many of the ARE-containing Nrf2-target genes are involved in: 1) maintaining the glutathione and thioredoxin antioxidant defence systems within the cell; 2) the detoxication of soft electrophiles and oxidants that could compromise cellular redox status and cellular function by modifying protein thiols; 3) the repair of damaged tissue. Thus in the presence of electrophiles or growth factors, Nrf2 increases expression of genes encoding proteins and enzymes that contribute to glutathione-based antioxidant defence (i.e. the cystine/glutamate transporter, glutamate-cysteine ligase catalytic and modifier subunits, glutaredoxin, glutathione peroxidase and glutathione reductase), thioredoxin-based antioxidant defence (i.e. peroxiredoxin, sulfiredoxin, thioredoxin and thioredoxin reductase), drug-metabolism [i.e. aldo-keto reductases, glutathione S-transferases, and NAD(P)H:quinone oxidoreductase-1 (NQO1)], drug efflux pumps [i.e. multidrug resistance-associated proteins] and cytoprotective proteins associated with heme and iron metabolism [i.e. biliverdin reductase, ferrochelatase, ferritin heavy and light, and heme oxygenase-1 (HMOX1)]. Induction of the above members of the ARE-gene

[1] Nrf1, Nrf2 and Nrf3 have also been called NF-E2 p45 like 1 (Nfe2l1), Nfe2l2 and Nfe2l3, respectively.

[2] The ARE has also been designated the electrophile response element (EpRE).

battery helps suppress the levels of reactive oxygen species and/or electrophiles in the cell and prevent oxidative stress [40, 41].

In addition to the well-documented role of Nrf2 in orchestrating adaptation to oxidative stress, increasing evidence indicates that Nrf2 also contributes to cell growth and the repair of damaged tissue [42-45]. In this case, activation of Nrf2 is presumed to facilitate cell growth by: 1) increasing nutrient availability; 2) augmenting the levels of NADPH, ATP and metabolic intermediates required for synthesis of macromolecules; 3) increasing production of signaling molecules; 4) limiting inflammation. Thus activation of Nrf2 results in the up-regulation of genes encoding transporters (i.e. the glucose transporter GLUT1, cystine/glutamate transporter SLC7A11, glycine transporter SLC6A9, and fatty acid translocase CD36) that are involved in the cellular uptake of glucose, amino acids and fatty acids. Also, activation of Nrf2 results in the up-regulation of enzymes involved in: carbohydrate metabolism (i.e. glucose-6-phosphate 1-dehydrogenase, isocitrate dehydrogenase 1, malic enzyme 1, 6-phosphogluconate dehydrogenase, transaldolase, and transketolase isoform 1) that regenerate NADPH; the β-oxidation of fatty acids (i.e. acetyl-CoA thioesterase, acetyl-CoA oxidase, carboxylesterase 1, and stearoyl-CoA desaturase) that produce ATP; purine nucleotide biosynthesis (i.e. phosphoribosyl pyrophosphate amidotransferase and methylenetetrahydrofolate dehydrogenase 2) that aid DNA synthesis. Moreover, Nrf2 also regulates directly Notch1 and augmenter of liver regeneration (ALR), both of which contribute to tissue repair [43, 45]. Taken together, the findings outlined above provide an important insight into the biochemical pathways by which activation of Nrf2 stimulates cell growth.

Molecular basis for the dual regulation of Nrf2 by Cullin-based ubiquitin ligases

A key feature of Nrf2 biology is that like other stress-responsive transcription factors it is a highly unstable protein because under normal homeostatic conditions it is continuously targeted for proteasomal degradation [46, 47]. The Nrf2 protein can be subdivided into seven regions, called Nrf2–ECH homology (Neh) domains 1-7. The intrinsic instability of Nrf2 can be attributed primarily to Neh2 and Neh6 as they contain several destruction motifs, whereas Neh1, Neh3, Neh4 and Neh5 are required for transactivation activity [41].

The first major repressor of Nrf2 to be discovered was Kelch-like ECH-associated protein-1 (Keap1), and it was identified using the Neh2 domain of Nrf2 as bait in a yeast two-hybrid screen [48]. It was initially thought that Keap1 sequesters Nrf2 in the cytoplasm, and that upon treatment with electrophiles the CNC-bZIP transcription factor is released by Keap1 to become free to translocate to the nucleus [48, 49]. Several models were advanced to explain the nuclear accumulation of Nrf2 following treatment with stressors: firstly, it was proposed that the release of Nrf2 from Keap1 required phosphorylation of Ser-40 in the Neh2 domain of Nrf2 by protein kinase C [50-52]; secondly, it was demonstrated that reactive Cys residues in a domain of Keap1 called the intervening region (IVR) serve as sensors for electrophiles and it was proposed that the chemical modification of these Cys residues triggered a conformational change in Keap1 that allowed release of Nrf2 [53]. Subsequently, several groups recognised that Keap1 targets Nrf2 for proteasomal degradation [54, 55], and several groups showed in close succession that Keap1 is a substrate adaptor for the Cullin-3–Rbx1 E3 ubiquitin ligase [56-59]. The mammalian Keap1 protein comprises 624 amino acids and contains at least 4 separate Cys-based stress sensors, in the IVR and elsewhere, that are triggered by reactive oxygen/nitrogen species and electrophiles [41]. It is a dimeric protein with two Kelch-repeat domains that each binds Nrf2 through either DLG or ETGE motifs in the Neh2 domain, and it seems improbable that Keap1 releases Nrf2 in a non-ubiquitylated state [60-62]. Indeed, the binding between Keap1 and Nrf2 is tighter when electrophilic agents modify the ubiquitin ligase substrate adaptor [63]. It seems likely that upon stress,

newly translated Nrf2 is able to bypass Keap1 [64], possibly because the latter protein is saturated with Nrf2 that is not ubiquitylated and degraded, and the modified Keap1 is ultimately degraded by autophagy [65].

The second major repressor of Nrf2 to be discovered was β-transducin repeat-containing protein (β-TrCP). Prior to the discovery, it was recognized that mutant Nrf2 lacking the high-affinity ETGE Keap1-interaction motif (i.e. $Nrf2^{\Delta ETGE}$-V5) was not particularly stable even though CRL^{Keap1} could not target the mutant protein for degradation. Systematic deletion mapping across the $Nrf2^{\Delta ETGE}$-V5 transcription factor identified amino acids bordering the N- and C-terminal boundaries of the Neh6 domain (i.e. residues 329-339 and 363-379 of mouse Nrf2) that conferred instability on the protein [66]. The N-terminal and C-terminal Neh6 regions contain the sequences DSGIS and DSAPGS, respectively, that resemble the consensus binding sequence for β-TrCP, a substrate adaptor for the S-phase kinase-associated protein-1 (Skp1)–Cullin-1–F-box ubiquitin ligase that is responsible for turnover of IκBα and β-catenin; the β-TrCP consensus site has been reported to be DSGϕXS [67, 68]. Whilst neither the DSGIS motif nor the DSAPGS motif conforms exactly to the β-TrCP consensus site, both have been found to recruit β-TrCP and support ubiquitylation of Nrf2 by $SCF^{\beta\text{-}TrCP}$ [69, 70]. Unlike the DLG and ETGE motifs in the Neh2 domain that are both required for CRL^{Keap1} ubiquitylation of Nrf2, the DSGIS and DSAPGS motifs in the Neh6 domain function independently of each other [70]. The reason for this is uncertain, as both Keap1 and β-TrCP are dimeric substrate adaptors, but one possibility is that whereas the Lys ubiquitin acceptor residues for CRL^{Keap1} lie between the DLG and ETGE motifs in Neh2, the Lys ubiquitin acceptor residues for $SCF^{\beta\text{-}TrCP}$ may lie outwith the region encompassed by the DSGIS and DSAPGS motifs.

Besides Keap1 and β-TrCP, Donna Zhang and colleagues have shown that Nrf2 is repressed by the E3 ubiquitin ligase Hrd1 (also called synoviolin) [71]. Hrd1 is activated in response to endoplasmic reticulum stress and it is therefore questionable whether is contributes to the repression of Nrf2 under normal homeostatic conditions. Importantly, however, activation of Hrd1 leads to loss of Nrf2 activity in cirrhotic liver and therapeutic targeting of Hdr1 in a murine model suppresses liver cirrhosis [71].

Glycogen synthase kinase-3 allows regulation of Nrf2 by growth factors

Many proteins that are ubiquitylated by $SCF^{\beta\text{-}TrCP}$ are themselves phosphorylated by glycogen synthase kinase-3 (GSK-3), including IκBα, β-catenin, Gli3 and securin [67, 68, 72, 73]. Salazar et al [74] first reported that GSK-3 inhibits the expression of Nrf2-target genes, and is able to phosphorylate the transcription factor. Evidence has subsequently been provided that GSK-3 phosphorylates Ser residues within the DSGIS motif [75, 76], and that this promotes ubiquitylation of Nrf2 by $SCF^{\beta\text{-}TrCP}$ [69, 70]. By contrast, the DSAPGS degron does not appear to be influenced by GSK-3 [70].

Unlike most kinases, GSK-3 is active in the cell under resting conditions, but is inhibited by phosphorylation of an N-terminal Ser residue (i.e. Ser-9 and Ser-21 in GSK-3β and GSK-3α, respectively) by protein kinase B (PKB)/Akt, and in turn PKB/Akt lies downstream of phosphoinositide 3-kinase (PI3K) [77]. As PI3K is closely associated with growth factor receptors and is intimately involved in cell growth and differentiation [78-80], it seems distinctly possible that the ability of epidermal growth factor, fibroblast growth factor, insulin, insulin-like growth factor, keratinocyte growth factor, nerve growth factor and platelet-derived growth factor to activate Nrf2 occurs through stimulation of the PI3K–PKB/Akt pathway and entails inhibition of GSK-3 and loss of repression by β-TrCP.

Regulation of Nrf2 by mitogen-activated protein kinases

Various cancer chemopreventive agents that induce Nrf2-target genes stimulate mitogen-activated protein kinase (MAPK) signaling, including extracellular signal-regulated protein kinases (ERKs), c-Jun N-terminal kinases (JNKs) and p38 kinases. Specifically, it has been reported that *tert*-butyl hydroquinone (tBHQ) activates ERK and $p38^{MAPK}$ [81-84], sulforaphane (SFN) activates ERK and can suppress activation of $p38^{MAPK}$ by aninomycin [82, 85], phenethyl isothiocyanate (PEITC) activates ERK and JNK [86, 87], dithiole-3-thione (D3T) activates ERK [88], and pyrrolidine dithiocarbamate (PDTC) activates ERK [89, 90]; see Figure 1 for structures. In the above studies, the use of kinase inhibitors and dominant-negative mutants to blunt gene induction suggest that ERK and JNK positively regulate Nrf2 activity. In marked contrast, $p38^{MAPK}$ has been reported to both positively and negatively regulate Nrf2 [84, 91-94] and this may reflect cell-specific differences in $p38^{MAPK}$ signaling pathways or response to chemicals.

Many of the investigators who examined the effects of MAPKs on Nrf2 activity considered that their activation resulted in phosphorylation of Nrf2 and that this event controlled translocation of the transcription factor from the cytoplasm to the nucleus. There are however at least three problems with this conclusion: firstly, it assumes the only mechanism of Nrf2 regulation is subcellular compartmentalization, and does not consider the impact of MAPK on transcriptional activation of the gene [95]; secondly, it does not consider the impact of MAPK on protein stability nor does it explain how knockdown or knockout of Keap1 is sufficient to induce Nrf2-target genes [96, 97]; thirdly, mutation of putative MAPK phosphorylation sites in Nrf2 have little impact on the activity of the transcription factor [90, 98]. The accumulated evidence suggests that both ERK and JNK positively regulate Nrf2 activity, but that their effects are probably indirect. Most, but not all studies, suggest that $p38^{MAPK}$ inhibits Nrf2, and again this appears to be indirect.

Regulation of Nrf2 by phosphatidylinositol-3-kinase

Over the years it has been found consistently that inhibition of PI3K by Wortmannin or LY294002 blunts Nrf2-mediated induction of ARE-driven genes. Jeff Johnson and colleagues first used LY294002 to diminish induction of NQO1 by tBHQ in IMR-32 human neuroblastoma cells [99], and they subsequently used microarray analysis of gene induction by tBHQ in IMR-32 cells and primary astrocytes from $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice to link PI3K to the induction of Nrf2-target genes [100, 101]. Around the same time, Antonio Cuadrado and colleagues showed that stimulation of PI3K affected Nrf2 indirectly by demonstrating first that activation of PI3K by nerve growth factor required activation of PKB/Akt in order for HMOX1 to be induced [32], and secondly that Nrf2 was required for HMOX1 induction [102]. Thereafter, Salazar et al [74] proposed that GSK-3 provides the link between activation of the PI3K–PKB/Akt pathway and stimulation of Nrf2-mediated gene induction in that they reported activation of PKB/Akt resulted in inhibitory phosphorylation of GSK-3, and that this resulted in failure by GSK-3 to phosphorylate Nrf2.

Regulation of Nrf2 by glycogen synthase kinase-3

As activation of PI3K and PKB/Akt cause inhibition of GSK-3α and GSK-3β by phosphorylation of their Ser-21 and Ser-9 residues, we hypothesized that chemopreventive agents might up-regulate Nrf2 by activating PI3K and PKB/Akt, thereby preventing formation of the DSGIS-containing phosphodegron in the Neh6 domain of Nrf2. To test this idea, we treated $Keap1^{-/-}$ mouse embryonic fibroblast (MEF) cells with tBHQ or SFN. As expected, we found that under normal conditions the Nrf2-target genes Nqo1 and Hmox1 were overexpressed in the $Keap1^{-/-}$ MEFs, relative to $Keap1^{+/+}$ MEFs. However, both Nqo1 and Hmox1 could be further induced by tBHQ, but not by SFN, in the mutant MEFs (**Figure 2**). Most importantly, induction of Nqo1 and Hmox1 by tBHQ in the $Keap1^{-/-}$ MEFs was

accompanied by inhibitory phosphorylation of GSK-3β at Ser-9 and by activating phosphorylation of PKB/Akt at Ser-473. Also of note, the PI3K inhibitor LY294002 markedly reduced Nrf2 protein levels as well as the basal expression of Nqo1 and Hmox1 in *Keap1*$^{-/-}$ MEFs, and blocked induction of Nqo1 and Hmox1 by tBHQ. These data suggest that PI3K positively regulates Nrf2, and support the hypothesis that tBHQ, but not SFN, activates PI3K and PKB/Akt.

Although it has not been clearly established how tBHQ stimulates PI3K, Mike Sporn and colleagues have shown that the semi-synthetic triterpenoid CDDO-Im, which is a potent inducer of Nrf2-target genes, can activate signaling downstream of PI3K by adducting to the catalytic Cys-124 residue of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) [104], the enzyme that cleaves the product of the PI3K reaction, phosphatidylinositol (3,4,5)-trisphosphate (PI3,4,5P_3 or PIP_3). Importantly, all Cys-dependent protein tyrosine phosphatases are redox sensitive [105]. We therefore propose that tBHQ either modifies Cys-124 in PTEN or another protein tyrosine phosphatase that impinges on PI3K signaling. We envisage that tBHQ and CDDO-Im increase PI3,4,5P_3 levels by inhibiting PTEN, and this causes activation of PDK1, which in turn activates PKB/Akt resulting in inhibition of GSK-3 and failure to produce the DSGIS-containing phosphodegron.

Besides influencing the stability of Nrf2 by catalyzing formation of the DSGIS-containing phosphodegron, it has been reported that GSK-3 indirectly controls the subcellular localization of Nrf2 because it lies upstream of Src non-receptor tyrosine kinases that phosphorylate mouse Nrf2 at Tyr-568 and human Nrf2 at Tyr-576. In this case, treatment of cells with H_2O_2 has been reported to result in phosphorylation of Nrf2 at Tyr-568/Tyr-576 by Fyn, Src, Yes and Fgr, which triggered nuclear export and degradation of the transcription factor [106, 107]. Further work is required to establish the precise pathway by which H_2O_2 activates the Src kinases and the putative involvement of GSK-3 in the process.

Concluding comments

Nrf2 plays an indispensible role in maintaining redox homeostasis, and it has become clear in recent years that it also controls cell growth. It is well known that Nrf2 activity is increased upon treatment of cells with electrophilic agents, because such chemicals antagonize the ability of Keap1 to direct the transcription factor to ubiquitylation by Cul3–Rbx1. It is however much less well appreciated that Nrf2 activity is controlled by growth factors. The body of literature we have reviewed suggests that GSK-3 plays a pivotal role in the regulation of Nrf2 by growth factors because it catalyzes formation of the DSGIS-containing phosphodegron that is recognised by $SCF^{\beta\text{-}TrCP}$. We have emphasized the ability of the PI3K–PKB/Akt pathway to inhibit GSK-3. However, as shown in **Figure 3**, other kinases besides PKB/Akt can inhibit GSK-3α/β by catalyzing phosphorylation of their Ser-21/9 residues, such as mTOR–$p70^{S6K}$, ERK–p90RSK, $p38^{MAPK}$ and PKC signaling pathways [77], suggesting other mechanisms by which Nrf2 can be regulated. It is intriguing that the kinases that potentially inhibit GSK-3 include ERK and PKC, which have been considered to be positive regulators of Nrf2 [50, 83, 84, 90]. It is also notable that GSK-3 has a strong preference for substrates that have already been phosphorylated by a 'priming' kinase at a Ser or Thr residue that is situated four or five residues to the C-terminal side of the amino acid that it phosphorylates [108]. It is not known whether Nrf2 has to be 'primed' before it can be modified by GSK-3, nor is it known whether induction of such a 'priming' kinase might serve as an alternative means of regulating Nrf2. Our preliminary investigations with peptide-based mini-protein assays suggest priming greatly enhances phosphorylation of Nrf2 by GSK-3. It is becoming clear that the regulation of Nrf2 stability and function is far more complex than just control by Keap1, and the input of multiple growth factor signaling pathways opens up an exciting new chapter in Nrf2 research and its association with human disease.

Acknowledgements
We are very grateful to Professor Masayuki Yamamoto for supplying Keap1-null MEFs, and to Professor Antonio Cuadrado for extremely helpful discussions. We thank Cancer Research UK for funding this work (C4909/A13786).

References

1. Amoutzias, G.D., Veron, A.S., Weiner, J. 3rd, Robinson-Rechavi, M., Bornberg-Bauer, E., Oliver, S.G. and Robertson, D.L. (2007) One billion years of bZIP transcription factor evolution: conservation and change in dimerization and DNA-binding site specificity. Mol. Biol. Evol. 24, 827-835.

2. Igarashi, K., Kataoka, K., Itoh, K., Hayashi, N., Nishizawa, M. and Yamamoto, M. (1994) Regulation of transcription by dimerization of erythroid factor NF-E2 p45 with small Maf proteins. Nature 367, 568-572.

3. Newman, J.R. and Keating, A.E. (2003) Comprehensive identification of human bZIP interactions with coiled-coil arrays. Science 300, 2097-2101.

4. Katsuoka, F., Motohashi, H., Ishii, T., Aburatani, H., Engel, J.D. and Yamamoto, M. Genetic evidence that small maf proteins are essential for the activation of antioxidant response element-dependent genes. (2005) Mol. Cell. Biol. 25, 8044-8051.

5. Andrews, N.C., Erdjument-Bromage, H., Davidson, M.B., Tempst, P. and Orkin, S.H. (1993) Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein. Nature 362, 722-728.

6. Kobayashi, A., Ito, E., Toki, T., Kogame, K., Takahashi, S., Igarashi, K., Hayashi, N. and Yamamoto, M. (1999) Molecular cloning and functional characterization of a new Cap'n' collar family transcription factor Nrf3. J. Biol. Chem. 274, 6443-6452.

7. McMahon, M., Itoh, K., Yamamoto, M., Chanas, S.A., Henderson, C.J., McLellan, L.I., Wolf, C.R., Cavin, C. and Hayes, J.D. (2001) The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. Cancer Res. 61, 3299-3307.

8. Sykiotis, G.P. and Bohmann, D. (2010) Stress-activated cap'n'collar transcription factors in aging and human disease. Sci. Signal. 3, re3.

9. Chevillard, G. and Blank, V. (2011) NFE2L3 (NRF3): the Cinderella of the Cap'n'Collar transcription factors. Cell. Mol. Life Sci. 68, 3337-3348.

10. Moi, P., Chan, K., Asunis, I., Cao, A. and Kan, Y.W. (1994) Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. Proc. Natl. Acad. Sci. USA. 91, 9926-9930.

11. Ramos-Gomez, M., Kwak, M.K., Dolan, P.M., Itoh, K., Yamamoto, M., Talalay, P and Kensler, T.W. (2001) Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. Proc. Natl. Acad. Sci. USA. 98, 3410-3415.

12. Chanas, S.A., Jiang, Q., McMahon, M., McWalter, G.K., McLellan, L.I., Elcombe, C.R., Henderson, C.J., Wolf, C.R., Moffat, G.J., Itoh, K., Yamamoto, M. and Hayes, J.D. (2002)

Loss of the Nrf2 transcription factor causes a marked reduction in constitutive and inducible expression of the glutathione S-transferase Gsta1, Gsta2, Gstm1, Gstm2, Gstm3 and Gstm4 genes in the livers of male and female mice. Biochem. J. 365, 405-416.

13. Motohashi, H. and Yamamoto, M. (2004) Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol. Med. 10, 549-557.

14. Clements, C.M., McNally, R.S., Conti, B.J., Mak, T.W. and Ting, J.P. (2006) DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. Proc. Natl. Acad. Sci. USA. 103, 15091-15096.

15. Kensler, T.W., Wakabayashi, N. and Biswal, S. (2007) Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu. Rev. Pharmacol. Toxicol. 47, 89-116.

16. Mitsuishi, Y., Taguchi, K., Kawatani, Y., Shibata, T., Nukiwa, T., Aburatani, H., Yamamoto, M. and Motohashi, H. (2012) Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer Cell. 22, 66-79.

17. Holmström, K.M., Baird, L., Zhang, Y., Hargreaves, I., Chalasani, A., Land, J.M., Stanyer, L., Yamamoto, M., Dinkova-Kostova, A.T. and Abramov, A.Y. (2013) Nrf2 impacts cellular bioenergetics by controlling substrate availability for mitochondrial respiration. Biol. Open. 2, 761-770.

18. Ludtmann, M.H., Angelova, P.R., Zhang, Y., Abramov, A.Y. and Dinkova-Kostova, A.T. (2014) Nrf2 affects the efficiency of mitochondrial fatty acid oxidation. Biochem J. 457, 415-424.

19. Meakin, P.J., Chowdhry, S., Sharma, R.S., Ashford, F.B., Walsh, S.V., McCrimmon, R.J., Dinkova-Kostova, A.T., Dillon, J.F., Hayes, J.D. and Ashford, M.L. (2014) Susceptibility of Nrf2-null mice to steatohepatitis and cirrhosis upon consumption of a high-fat diet is associated with oxidative stress, perturbation of the unfolded protein response, and disturbance in the expression of metabolic enzymes but not with insulin resistance. Mol. Cell. Biol. 34, 3305-3320.

20. Nioi, P., McMahon, M., Itoh, K., Yamamoto, M. and Hayes, J.D. (2003) Identification of a novel Nrf2-regulated antioxidant response element (ARE) in the mouse NAD(P)H:quinone oxidoreductase 1 gene: reassessment of the ARE consensus sequence. Biochem. J. 374, 337-348.

21. Prestera, T., Holtzclaw, W.D., Zhang, Y. and Talalay, P. (1993) Chemical and molecular regulation of enzymes that detoxify carcinogens. Proc. Natl. Acad. Sci. USA. 90, 2965-2969.

22. Dinkova-Kostova, A.T., Fahey, J.W. and Talalay, P. (2004) Chemical structures of inducers of nicotinamide quinone oxidoreductase 1 (NQO1). Methods Enzymol. 382, 423-448.

23. Hayes, J.D., McMahon, M., Chowdhry, S. and Dinkova-Kostova, A.T. (2010) Cancer chemoprevention mechanisms mediated through the Keap1-Nrf2 pathway. Antioxid. Redox Signal. 13, 1713-1748.

24. Papaiahgari, S., Zhang, Q., Kleeberger, S.R., Cho, H.Y. and Reddy, S.P. (2006) Hyperoxia stimulates an Nrf2-ARE transcriptional response via ROS-EGFR-PI3K-Akt/ERK MAP kinase signaling in pulmonary epithelial cells. Antioxid. Redox Signal. 8, 43-52.

25. Yamadori, T., Ishii, Y., Homma, S., Morishima, Y., Kurishima, K., Itoh, K., Yamamoto, M., Minami, Y., Noguchi, M. and Hizawa, N. (2012) Molecular mechanisms for the regulation of Nrf2-mediated cell proliferation in non-small-cell lung cancers. Oncogene 31, 4768-4777.

26. Vargas, M.R., Pehar, M., Cassina, P., Martínez-Palma, L., Thompson, J.A., Beckman, J.S. and Barbeito, L. (2005) Fibroblast growth factor-1 induces heme oxygenase-1 via nuclear factor erythroid 2-related factor 2 (Nrf2) in spinal cord astrocytes: consequences for motor neuron survival. J. Biol. Chem. 280, 25571-25579.

27. Harrison, E.M., McNally, S.J., Devey, L., Garden, O.J., Ross, J.A. and Wigmore, S.J. (2006) Insulin induces heme oxygenase-1 through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in renal cells. FEBS J. 273, 2345-2356.

28. Geraldes, P., Yagi, K., Ohshiro, Y., He, Z., Maeno, Y., Yamamoto-Hiraoka, J., Rask-Madsen, C., Chung, S.W., Perrella, M.A. and King, G.L. (2008) Selective regulation of heme oxygenase-1 expression and function by insulin through IRS1/phosphoinositide 3-kinase/Akt-2 pathway. J. Biol. Chem. 283, 34327-34336.

29. Kim, Y., Li, E. and Park, S. (2012) Insulin-like growth factor-1 inhibits 6-hydroxydopamine-mediated endoplasmic reticulum stress-induced apoptosis via regulation of heme oxygenase-1 and Nrf2 expression in PC12 cells. Int. J. Neurosci. 122, 641-649.

30. Braun, S., Hanselmann, C., Gassmann, M.G., auf dem Keller, U., Born-Berclaz, C., Chan, K., Kan, Y.W. and Werner, S. (2002) Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound. Mol. Cell. Biol. 22, 5492-5505.

31. Chowdhury, I., Fisher, A.B., Christofidou-Solomidou, M., Gao, L., Tao, J.Q., Sorokina, E.M., Lien, Y.C., Bates, S.R. and Feinstein, S.I. (2014) Keratinocyte growth factor and glucocorticoid induction of human peroxiredoxin 6 gene expression occur by independent mechanisms that are synergistic. Antioxid. Redox Signal. 20, 391-402.

32. Salinas, M., Diaz, R., Abraham, N.G., Ruiz de Galarreta, C.M. and Cuadrado, A. (2003) Nerve growth factor protects against 6-hydroxydopamine-induced oxidative stress by increasing expression of heme oxygenase-1 in a phosphatidylinositol 3-kinase-dependent manner. J. Biol. Chem. 278, 13898-13904.

33. Tohidnezhad, M., Wruck, C.J., Slowik, A., Kweider, N., Beckmann, R., Bayer, A., Houben, A., Brandenburg, L.O., Varoga, D., Sönmez, T.T., Stoffel, M., Jahr, H., Lippross, S. and Pufe, T. (2014) Role of platelet-released growth factors in detoxification of reactive oxygen species in osteoblasts. Bone 65, 9-17.

34. Heiss, E.H., Schachner, D., Zimmermann, K. and Dirsch, V.M. (2013) Glucose availability is a decisive factor for Nrf2-mediated gene expression. Redox Biol. 1, 359-365.

35. Kwak, M.K., Wakabayashi, N., Itoh, K., Motohashi, H., Yamamoto, M. and Kensler, T.W. (2003) Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. J. Biol. Chem. 278, 8135-8145.

36. MacLeod, A.K., McMahon, M., Plummer, S.M., Higgins, L.G., Penning, T.M., Igarashi, K. and Hayes, J.D. (2009) Characterization of the cancer chemopreventive NRF2-dependent gene battery in human keratinocytes: demonstration that the KEAP1-NRF2 pathway, and not the BACH1-NRF2 pathway, controls cytoprotection against electrophiles as well as redox-cycling compounds. Carcinogenesis 30, 1571-1580.

37. Agyeman, A.S., Chaerkady, R., Shaw, P.G., Davidson, N.E., Visvanathan, K., Pandey, A. and Kensler, T.W. (2012) Transcriptomic and proteomic profiling of KEAP1 disrupted and sulforaphane-treated human breast epithelial cells reveals common expression profiles. Breast Cancer Res. Treat. 132, 175-187.

38. Chorley, B.N., Campbell, M.R., Wang, X., Karaca, M., Sambandan, D., Bangura, F., Xue, P., Pi, J., Kleeberger, S.R. and Bell, D.A. (2012) Identification of novel NRF2-regulated genes by ChIP-Seq: influence on retinoid X receptor alpha. Nucleic Acids Res. 40, 7416-7429.

39. Hirotsu, Y., Katsuoka, F., Funayama, R., Nagashima, T., Nishida, Y., Nakayama, K., Engel, J.D. and Yamamoto, M. (2012) Nrf2-MafG heterodimers contribute globally to antioxidant and metabolic networks. Nucleic Acids Res. 40, 10228-10239.

40. Higgins, L.G., Kelleher, M.O., Eggleston, I.M., Itoh, K., Yamamoto, M. and Hayes, J.D. (2009) Transcription factor Nrf2 mediates an adaptive response to sulforaphane that protects fibroblasts in vitro against the cytotoxic effects of electrophiles, peroxides and redox-cycling agents. Toxicol. Appl. Pharmacol. 237, 267-280.

41. Hayes, J.D. and Dinkova-Kostova, A.T. (2014) The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem. Sci. 39, 199-218.

42. Beyer, T.A., Xu, W., Teupser, D., auf dem Keller, U., Bugnon, P., Hildt, E., Thiery, J., Kan, Y.W. and Werner, S. (2008) Impaired liver regeneration in Nrf2 knockout mice: role of ROS-mediated insulin/IGF-1 resistance. EMBO J. 27, 212-223.

43. Wakabayashi, N., Shin, S., Slocum, S.L., Agoston, E.S., Wakabayashi, J., Kwak, M.K., Misra, V., Biswal, S., Yamamoto, M. and Kensler, T.W. (2010) Regulation of notch1 signaling by nrf2: implications for tissue regeneration. Sci. Signal. 3, ra52.

44. Dayoub, R., Vogel, A., Schuett, J., Lupke, M., Spieker, S.M., Kettern, N., Hildt, E., Melter, M. and Weiss, T.S. (2013) Nrf2 activates augmenter of liver regeneration (ALR) via antioxidant response element and links oxidative stress to liver regeneration. Mol. Med. 19, 237-244.

45. Al-Sawaf, O., Fragoulis, A., Rosen, C., Keimes, N., Liehn, E.A., Hölzle, F., Kan, Y.W., Pufe, T., Sönmez, T.T. and Wruck, C.J. (2014) Nrf2 augments skeletal muscle regeneration after ischaemia-reperfusion injury. J. Pathol. 234, 538-547.

46. Nguyen, T., Sherratt, P.J., Huang, H.C., Yang, C.S. and Pickett, C.B. (2003) Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element. Degradation of Nrf2 by the 26 S proteasome. J. Biol. Chem. 278, 4536-4541.

47. Stewart, D., Killeen, E., Naquin, R., Alam, S. and Alam, J. (2003) Degradation of transcription factor Nrf2 via the ubiquitin-proteasome pathway and stabilization by cadmium. J. Biol. Chem. 278, 2396-2402.

48. Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J.D. and Yamamoto, M. (1999) Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 13, 76-86.

49. Zipper, L.M. and Mulcahy, R.T. (2002) The Keap1 BTB/POZ dimerization function is required to sequester Nrf2 in cytoplasm. J. Biol. Chem. 277, 36544-36552.

50. Huang, H.C., Nguyen, T. and Pickett, C.B. (2002) Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. J. Biol. Chem. 277, 42769-42774.

51. Numazawa, S., Ishikawa, M., Yoshida, A., Tanaka, S. and Yoshida, T. (2003) Atypical protein kinase C mediates activation of NF-E2-related factor 2 in response to oxidative stress. Am. J. Physiol. Cell. Physiol. 285, C334-342.

52. Bloom, D.A. and Jaiswal, A.K. (2003) Phosphorylation of Nrf2 at Ser40 by protein kinase C in response to antioxidants leads to the release of Nrf2 from INrf2, but is not required for Nrf2 stabilization/accumulation in the nucleus and transcriptional activation of antioxidant response element-mediated NAD(P)H:quinone oxidoreductase-1 gene expression. J. Biol. Chem. 278, 44675-44682.

53. Dinkova-Kostova, A.T., Holtzclaw, W.D., Cole, R.N., Itoh, K., Wakabayashi, N., Katoh, Y., Yamamoto, M. and Talalay, P. (2002) Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc. Natl. Acad. Sci. USA. 99, 11908-11913.

54. McMahon, M., Itoh, K., Yamamoto, M. and Hayes, J.D. (2003) Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. J. Biol. Chem. 278, 21592-21600.

55. Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., O'Connor, T. and Yamamoto, M. (2003) Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. Genes Cells. 8, 379-391.

56. Kobayashi, A., Kang, M.I., Okawa, H., Ohtsuji, M., Zenke, Y., Chiba, T., Igarashi, K. and Yamamoto, M. (2004) Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol. Cell. Biol. 24, 7130-7139.

57. Cullinan, S.B., Gordan, J.D., Jin, J., Harper, J.W. and Diehl, J.A. (2004) The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. Mol. Cell. Biol. 24, 8477-8486.

58. Zhang, D.D., Lo, S.C., Cross, J.V., Templeton, D.J. and Hannink, M. (2004) Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. Mol. Cell. Biol. 24, 10941-10953.

59. Furukawa, M. and Xiong, Y. (2005) BTB protein Keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin 3-Roc1 ligase. Mol. Cell. Biol. 25, 162-171.

60. Eggler, A.L., Liu, G., Pezzuto, J.M., van Breemen, R.B. and Mesecar, A.D. (2005) Modifying specific cysteines of the electrophile-sensing human Keap1 protein is insufficient to disrupt binding to the Nrf2 domain Neh2. Proc. Natl. Acad. Sci. USA. 102, 10070-10075.

61. Tong, K.I., Katoh, Y., Kusunoki, H., Itoh, K., Tanaka, T. and Yamamoto, M. (2006) Keap1 recruits Neh2 through binding to ETGE and DLG motifs: characterization of the two-site molecular recognition model. Mol. Cell. Biol. 26, 2887-2900.

62. McMahon, M., Thomas, N., Itoh, K., Yamamoto, M. and Hayes, J.D. (2006) Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a "tethering" mechanism: a two-site interaction model for the Nrf2-Keap1 complex. J. Biol. Chem. 281, 24756-24768.

63. Baird, L., Llères, D., Swift, S. and Dinkova-Kostova, A.T. (2013) Regulatory flexibility in the Nrf2-mediated stress response is conferred by conformational cycling of the Keap1-Nrf2 protein complex. Proc. Natl. Acad. Sci. USA. 110, 15259-15264.

64. Kobayashi, A., Kang, M.I., Watai, Y., Tong, K.I., Shibata, T., Uchida, K. and Yamamoto, M. (2006) Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. Mol. Cell. Biol. 26, 221-219.

65. Taguchi, K., Fujikawa, N., Komatsu, M., Ishii, T., Unno, M., Akaike, T., Motohashi, H. and Yamamoto, M. (2012) Keap1 degradation by autophagy for the maintenance of redox homeostasis. Proc. Natl. Acad. Sci. USA. 109, 13561-13566.

66. McMahon, M., Thomas, N., Itoh, K., Yamamoto, M. and Hayes, J.D. (2004) Redox-regulated turnover of Nrf2 is determined by at least two separate protein domains, the redox-sensitive Neh2 degron and the redox-insensitive Neh6 degron. J. Biol. Chem. 279, 31556-31567.

67. Aberle, H., Bauer, A., Stappert, J., Kispert, A. and Kemler, R. (1997) beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J. 16, 3797-3804.

68. Winston, J.T., Strack, P., Beer-Romero, P., Chu, C.Y., Elledge, S.J. and Harper, J.W. (1999) The SCFbeta-TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IkappaBalpha and beta-catenin and stimulates IkappaBalpha ubiquitination in vitro. Genes Dev. 13, 270-283.

69. Rada, P., Rojo, A.I., Chowdhry, S., McMahon, M., Hayes, J.D. and Cuadrado, A. (2011) SCF/{beta}-TrCP promotes glycogen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. Mol. Cell. Biol. 31, 1121-1133.

70. Chowdhry, S., Zhang, Y., McMahon, M., Sutherland, C., Cuadrado, A. and Hayes, J.D. (2013) Nrf2 is controlled by two distinct β-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. Oncogene 32, 3765-3781.

71. Wu, T., Zhao, F., Gao, B., Tan, C., Yagishita, N., Nakajima, T., Wong, P.K., Chapman, E., Fang, D. and Zhang, D.D. (2014) Hrd1 suppresses Nrf2-mediated cellular protection during liver cirrhosis. Genes Dev. 28, 708-722.

72. Tempé, D., Casas, M., Karaz, S., Blanchet-Tournier, M.F. and Concordet, J.P. (2006) Multisite protein kinase A and glycogen synthase kinase 3beta phosphorylation leads to Gli3 ubiquitination by SCFbetaTrCP. Mol. Cell. Biol. 26, 4316-4326.

73. Limón-Mortés, M.C., Mora-Santos, M., Espina, A., Pintor-Toro, J.A., López-Román, A., Tortolero, M. and Romero, F. (2008) UV-induced degradation of securin is mediated by SKP1-CUL1-beta TrCP E3 ubiquitin ligase. J. Cell. Sci. 121, 1825-1831.

74. Salazar, M., Rojo, A.I., Velasco, D., de Sagarra, R.M. and Cuadrado, A. (2006) Glycogen synthase kinase-3beta inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. J. Biol. Chem. 281, 14841-14851.

75. Rada, P., Rojo, A.I., Evrard-Todeschi, N., Innamorato, N.G., Cotte, A., Jaworski, T., Tobón-Velasco, J.C., Devijver, H., García-Mayoral, M.F., Van Leuven, F., Hayes, J.D., Bertho, G. and Cuadrado, A. (2012) Structural and functional characterization of Nrf2 degradation by the glycogen synthase kinase 3/β-TrCP axis. Mol. Cell. Biol. 32, 3486-3499.

76. Rojo, A.I., Rada, P., Mendiola, M., Ortega-Molina, A., Wojdyla, K., Rogowska-Wrzesinska, A., Hardisson, D., Serrano, M. and Cuadrado, A. (2014) The PTEN/NRF2 Axis Promotes Human Carcinogenesis. Antioxid. Redox Signal. 21, 2498-2514.

77. Kaidanovich-Beilin, O. and Woodgett, J.R. (2011) GSK-3: Functional Insights from Cell Biology and Animal Models. Front Mol Neurosci. 4, 40.

78. Liu, P., Cheng, H., Roberts, T.M. and Zhao, J.J. (2009) Targeting the phosphoinositide 3-kinase pathway in cancer. Nat. Rev. Drug Discov. 8, 627-644.

79. Vanhaesebroeck, B., Guillermet-Guibert, J., Graupera, M. and Bilanges, B. (2010) The emerging mechanisms of isoform-specific PI3K signalling. Nat. Rev. Mol. Cell. Biol. 11, 329-341.

80. Thorpe, L.M., Yuzugullu, H. and Zhao, J.J. (2014) PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. Nat. Rev. Cancer 15, 7-24.

81. Yu, R., Tan, T.H. and Kong, A.N. (1997) Butylated hydroxyanisole and its metabolite tert-butylhydroquinone differentially regulate mitogen-activated protein kinases. The role of oxidative stress in the activation of mitogen-activated protein kinases by phenolic antioxidants. J. Biol. Chem. 272, 28962-28970.

82. Yu, R., Lei, W., Mandlekar, S., Weber, M.J., Der, C.J., Wu, J. and Kong, A.N. (1999) Role of a mitogen-activated protein kinase pathway in the induction of phase II detoxifying enzymes by chemicals. J. Biol. Chem. 274, 27545-27552.

83. Yu, R., Chen, C., Mo, Y.Y., Hebbar, V., Owuor, E.D., Tan, T.H. and Kong, A.N. (2000) Activation of mitogen-activated protein kinase pathways induces antioxidant response element-mediated gene expression via a Nrf2-dependent mechanism. J. Biol. Chem. 275, 39907-39913.

84. Yu, R., Mandlekar, S., Lei, W., Fahl, W.E,. Tan, T.H. and Kong, A.N. (2000) p38 mitogen-activated protein kinase negatively regulates the induction of phase II drug-metabolizing enzymes that detoxify carcinogens. J. Biol. Chem. 275, 2322-2327.

85. Keum, Y.S., Yu, S., Chang, P.P., Yuan, X., Kim, J.H., Xu, C., Han, J., Agarwal, A. and Kong, A.N. (2006) Mechanism of action of sulforaphane: inhibition of p38 mitogen-activated protein kinase isoforms contributing to the induction of antioxidant response element-mediated heme oxygenase-1 in human hepatoma HepG2 cells. Cancer Res. 66, 8804-8813.

86. Keum, Y.S., Owuor, E.D., Kim, B.R., Hu, R. and Kong, A.N. (2003) Involvement of Nrf2 and JNK1 in the activation of antioxidant responsive element (ARE) by chemopreventive agent phenethyl isothiocyanate (PEITC). Pharm. Res. 20, 1351-1356.

87. Xu, C., Yuan, X., Pan, Z., Shen, G., Kim, J.H., Yu, S., Khor, T.O., Li, W., Ma, J. and Kong, A.N. (2006) Mechanism of action of isothiocyanates: the induction of ARE-regulated genes is associated with activation of ERK and JNK and the phosphorylation and nuclear translocation of Nrf2. Mol. Cancer Ther. 5, 1918-1926.

88. Manandhar, S., Cho, J.M., Kim, J.A., Kensler, T.W. and Kwak, M.K. (2007) Induction of Nrf2-regulated genes by 3H-1, 2-dithiole-3-thione through the ERK signaling pathway in murine keratinocytes. Eur. J. Pharmacol. 577, 17-27.

89. Zipper, L.M. and Mulcahy, R.T. (2000) Inhibition of ERK and p38 MAP kinases inhibits binding of Nrf2 and induction of GCS genes. Biochem. Biophys. Res. Commun. 278, 484-492.

90. Zipper, L.M. and Mulcahy, R.T. (2003) Erk activation is required for Nrf2 nuclear localization during pyrrolidine dithiocarbamate induction of glutamate cysteine ligase modulatory gene expression in HepG2 cells. Toxicol. Sci. 73, 124-134.

91. Alam, J., Wicks, C., Stewart, D., Gong, P., Touchard, C., Otterbein, S., Choi, A.M., Burow, M.E. and Tou, J. (2000) Mechanism of heme oxygenase-1 gene activation by cadmium in MCF-7 mammary epithelial cells. Role of p38 kinase and Nrf2 transcription factor. J. Biol. Chem. 275, 27694-27702.

92. Keum, Y.S., Yu, S., Chang, P.P., Yuan, X., Kim, J.H., Xu, C., Han, J., Agarwal, A. and Kong, A.N. (2006) Mechanism of action of sulforaphane: inhibition of p38 mitogen-activated protein kinase isoforms contributing to the induction of antioxidant response element-mediated heme oxygenase-1 in human hepatoma HepG2 cells. Cancer Res. 66, 8804-8813.

93. Naidu, S., Vijayan, V., Santoso, S., Kietzmann, T. and Immenschuh, S. (2009) Inhibition and genetic deficiency of p38 MAPK up-regulates heme oxygenase-1 gene expression via Nrf2. J. Immunol. 182, 7048-7057.

94. Tsai, C.W., Lin, C.Y. and Wang, Y.J. (2011) Carnosic acid induces the NAD(P)H: quinone oxidoreductase 1 expression in rat clone 9 cells through the p38/nuclear factor erythroid-2 related factor 2 pathway. J. Nutr. 141, 2119-2125.

95. DeNicola, G.M., Karreth, F.A., Humpton, T.J., Gopinathan, A., Wei, C., Frese, K., Mangal, D., Yu, K.H., Yeo, C.J., Calhoun, E.S., Scrimieri, F., Winter, J.M., Hruban, R.H., Iacobuzio-Donahue, C., Kern, S.E., Blair, I.A. and Tuveson, D.A. (2011) Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature 475, 106-109.

96. Wakabayashi, N., Itoh, K., Wakabayashi, J., Motohashi, H., Noda, S., Takahashi, S., Imakado, S., Kotsuji, T., Otsuka, F., Roop, D.R., Harada, T., Engel, J.D. and Yamamoto, M. (2003) Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. Nat. Genet. 35, 238-245.

97. Devling, T.W., Lindsay, C.D., McLellan, L. I., McMahon, M. and Hayes, J.D. (2005) Utility of siRNA against Keap1 as a strategy to stimulate a cancer chemopreventive phenotype. Proc. Natl. Acad. Sci. USA. 102, 7280-7285A.

98. Sun, Z., Huang, Z. and Zhang, D.D. (2009) Phosphorylation of Nrf2 at multiple sites by MAP kinases has a limited contribution in modulating the Nrf2-dependent antioxidant response. PLoS One. 4, e6588.

99. Lee, J.M., Hanson, J.M., Chu, W.A. and Johnson, J.A. (2001) Phosphatidylinositol 3-kinase, not extracellular signal-regulated kinase, regulates activation of the antioxidant-responsive element in IMR-32 human neuroblastoma cells. J. Biol. Chem. 276, 20011-20016.

100. Li, J., Lee, J.M. and Johnson, J.A. (2002) Microarray analysis reveals an antioxidant responsive element-driven gene set involved in conferring protection from an oxidative stress-induced apoptosis in IMR-32 cells. J. Biol. Chem. 277, 388-394.

101. Lee, J.M., Calkins, M.J., Chan, K., Kan, Y.W. and Johnson, J.A. (2003) Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. J. Biol. Chem. 278, 12029-12038.

102. Martin, D., Rojo, A.I., Salinas, M., Diaz, R., Gallardo, G., Alam, J., De Galarreta, C.M. and Cuadrado, A. (2004) Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol. J. Biol. Chem. 279, 8919-8929.

103. Fahey, J.W., Dinkova-Kostova, A.T., Stephenson, K.K. and Talalay, P. (2004) The "Prochaska" microtiter plate bioassay for inducers of NQO1. Methods Enzymol. 382, 243-258.

104. Pitha-Rowe, I., Liby, K., Royce, D. and Sporn, M. (2009) Synthetic triterpenoids attenuate cytotoxic retinal injury: cross-talk between Nrf2 and PI3K/AKT signaling through inhibition of the lipid phosphatase PTEN. Invest. Ophthalmol. Vis. Sci. 50, 5339-5347.

105. Ross, S.H., Lindsay, Y., Safrany, S.T., Lorenzo, O., Villa, F., Toth, R., Clague, M.J., Downes, C.P. and Leslie, N.R. (2007) Differential redox regulation within the PTP superfamily. Cell Signal. 19, 1521-1530.

106. Jain, A.K. and Jaiswal, A.K. (2007) GSK-3beta acts upstream of Fyn kinase in regulation of nuclear export and degradation of NF-E2 related factor 2. J. Biol. Chem. 282, 16502-16510.

107. Niture, S.K., Jain, A.K., Shelton, P.M. and Jaiswal, A.K. (2011) Src subfamily kinases regulate nuclear export and degradation of transcription factor Nrf2 to switch off Nrf2-mediated antioxidant activation of cytoprotective gene expression. J. Biol. Chem. 286, 28821-28832.

108. Cohen, P. and Frame, S. (2001) The renaissance of GSK3. Nat. Rev. Mol. Cell. Biol. 2, 769-776.

Figure 1. Chemical structures of electrophilic agents that induce Nrf2-target genes. The structures are (i) *tert*-butyl hydroquinone; (ii) sulforaphane; (iii) phenethyl isothiocyanate; (iv) dithiole-3-thione; (v) pyrrolidine dithiocarbamate; (vi) 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid; (vii) bardoxolone methyl.

Figure 2. Keap1-independent induction of Nrf2-target genes by *tert*-butyl hydroquinone but not by sulforaphane. Duplicate sets of 60-mm petri-dishes of wild-type and *Keap1*$^{-/-}$ MEFs, plated in DMEM medium containing 10% FBS, were grown in DMEM containing low serum (0.1% FBS) for 16 h. Thereafter, they were transferred to fresh medium containing 0.2% FBS and treated with tBHQ, SFN or vehicle control for various time intervals. In some instances, the fibroblasts were pre-treated for 30 min with 10 μM LY294002 immediately prior to transfer to fresh medium and treatment with tBHQ or SFN. **(A)** Whole cell lysates were prepared from *Keap1*$^{-/-}$ MEFs that had been treated for 18 h with tBHQ, SFN or DMSO vehicle control. Thereafter, Nqo1 enzyme activity was measured in the 10,000 x *g* supernatant by the 'Prochaska' bioassay method [103]. Data obtained from the MEFs that had been pretreated with LY294002 are shown in solid bars. Results that were significantly higher than the DMSO vehicle control with p-values of 0.01-0.001 or <0.001 are indicated with double (**) or triple (***) asterisk signs, respectively; ns = not significant. **(B)** Nqo1 mRNA levels were measured in *Keap1*$^{-/-}$ MEFs that had been treated for 12 h with tBHQ, SFN or DMSO. Data from MEFs that had been pretreated with 10 μM LY294002 prior to treatment with tBHQ or SFN are shown in solid bars. **(C)** MEFs from *Keap1*$^{-/-}$ and *Keap1*$^{+/+}$ mice were treated with tBHQ, SFN or DMSO for 2 h, either with or without pretreatment with LY294002. Whole cell lysates were probed with antibodies specific for Nrf2, Nqo1, Hmox1, phospho-Ser473 Akt (i.e. activated PKB/Akt), total Akt, phospho-Ser9 GSK-3β (i.e. inactive GSK-3β), total GSK-3, and Gapdh.

Figure 3. Regulation of Nrf2 by phosphorylation of its Neh6 domain. Evidence indicates that GSK-3 negatively controls Nrf2 by phosphorylating the DSGIS motif in its Neh6 domain and promoting degradation via the actions of SCF$^{\beta\text{-TrCP}}$. As indicated in **(1)**, shown on mid right-hand side, phosphorylation of most validated substrates by GSK-3 requires 'priming' (i.e. pre-phosphorylation) by an alternative kinase. Therefore Nrf2 degradation could be regulated by control of 'priming'. As indicated in **(2)**, shown on mid left-hand side, GSK-3α/β is itself inhibited by phosphorylation of an N-terminal Ser-21/9 residue. The main kinases reported to date to catalyze inhibitory phosphorylation of GSK-3 are members of the AGC class of Kinases (including PKB/Akt, PKC and p70^{S6K}). Moreover, p90^{RSK} and p38^{MAPK} have also been reported to inhibit GSK-3. Therefore, as indicated in **(3)**, shown in upper half, activation of PKB/Akt, p70^{S6K}, p90^{RSK}, p38^{MAPK} and PKC could inhibit GSK-3 and reduce Neh-6 phosphorylation and Nrf2 degradation.